

### **AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listing, of claims in the application.

#### **Listing of Claims:**

1 (Withdrawn). A method for treating a specimen of semen containing sperm cells to increase the relative number of sperm cells of a selected sex type in a treated specimen to increase the potential for conceiving an offspring of the selected sex, the method comprising holding the specimen at a predetermined temperature for at least a predetermined time and, thereafter, treating the specimen of semen to increase the relative ability of a at least a portion of the semen to conceive an offspring of the desired sex.

2 (Withdrawn). The method of claim 1, wherein the treating step comprises contacting the sperm cells with an agent that preferentially effects sperm cells of a selected sex type.

3 (Withdrawn). A method for treating a specimen of semen containing sperm cells to increase the relative number of sperm cells of a selected sex type in a treated specimen to increase the potential for conceiving an offspring of the selected sex, the method comprising separating the semen into two components comprising a first component having a higher number of sperm of the selected sex type than sperm of a non selected sex type and a second component having a higher number of sperm of the non selected sex type relative to sperm of the selected sex type, wherein the separating step is performed after a predetermined percent of the sperm cells exhibit a punctate pattern, which is capable of determination by labeling the sperm cells with Koo antibody and determining the percent of cells labeled.

4 (Withdrawn). The method of claim 3, wherein the separating step comprises contacting the sperm with a cell binding agent; permitting the sperm of the non selected sex type to preferentially bind to the cell binding agent, and separating the cell binding



agent with preferentially bound sperm of the non selected sex type from non bound sperm, thereby providing said first component.

5 (Withdrawn). The method of claim 3, wherein the first component comprises a higher number of X-chromosome bearing sperm relative to Y- chromosome bearing sperm and the second component comprises a higher number of Y- chromosome bearing sperm relative to X- chromosome bearing sperm.

6 (Withdrawn). The method of claim 3, wherein the separating step is performed after 20 % of the sperm cells exhibit the punctate pattern.

7 (Withdrawn). The method of claim 3, further comprising cooling the semen before performing the separating step.

8 (Withdrawn). The method of claim 3, further comprising collecting the semen and immediately cooling the semen.

9 (Withdrawn). The method of claim 8, wherein the semen is cooled to at least about 16°C.

10 (Withdrawn). The method of claim 8, wherein the semen is cooled to at least about 12°C.

11 (Withdrawn). A method for increasing the probability of producing an offspring of a mammalian species having a selected sex, said method comprising: separating semen containing sperm cells of the mammalian species into two components comprising a first component having a higher number of sperm of the selected sex type than sperm of a non selected sex type and a second component having a higher number of sperm of the non selected sex type relative to sperm of the selected sex type, wherein the separating step is performed after a predetermined percent of the sperm cells exhibit a punctate pattern, which is capable of determination by labeling the



sperm cells with Koo antibody, and fertilizing an egg of the mammalian species with the first component.

12 (Withdrawn). The method of claim 11, wherein the separating step comprises contacting the sperm with a cell binding agent; permitting the sperm of the non selected sex type to preferentially bind to the cell binding agent, and separating the cell binding agent with preferentially bound sperm of the non selected sex type from non bound sperm, thereby providing said first component.

13 (Withdrawn). The method of claim 11, wherein the first component comprises a higher number of X-chromosome bearing sperm relative to Y- chromosome bearing sperm and the second component comprises a higher number of Y- chromosome bearing sperm relative to X- chromosome bearing sperm.

14 (Withdrawn). The method of claim 11, wherein the separating step is performed after 20 % of the sperm cells exhibit the punctate pattern.

15 (Withdrawn). The method of claim 11, further comprising cooling the semen before performing the separating step.

16 (Withdrawn). The method of claim 11, further comprising collecting the semen and immediately cooling the semen.

17 (Withdrawn). The method of claim 16, wherein the semen is cooled to at least about 16°C.

18 (Withdrawn). The method of claim 16, wherein the semen is cooled to at least about 12°C.

19 (Withdrawn). The method of claim 11, wherein the first component contains two-thirds viable X- chromosome bearing sperm and one-third viable Y - chromosome bearing sperm.



20 (Withdrawn). The method of claim 11, wherein twice as many female calves are born than male calves when the treated semen is used to fertilize a plurality of eggs.

21 (Withdrawn). A method for separating a selected population of cells from a sample of semen, the method comprising:

cooling the semen to a predetermined temperature;

waiting until a predetermined percent of the cells exhibit a punctate pattern, which is capable of determination by labeling the sperm cells with Koo antibody;

contacting the sample with a cell binding agent to preferentially bind to the selected population of cells for a time sufficient for the cell binding agent to bind the selected cells; and

separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells.

22 (Withdrawn). The method of claim 21, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting preferred sex type is at least about 10% greater than a second number of cells exhibiting a non preferred sex type.

23 (Withdrawn). The method of claim 21, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 20% greater than a second number of cells exhibiting a non preferred sex type.

24 (Withdrawn). The method of claim 21, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 50% greater than a second number of cells exhibiting a non preferred sex type.

25 (Withdrawn). The method of claim 21, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at



least about 100% greater than a second number of cells exhibiting a non preferred desired sex type.

26 (Withdrawn). The method of claim 21, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

27 (Withdrawn). The method of claim 21, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.

28 (Withdrawn). A method of insemination, the method comprising obtaining semen containing a population of spermatozoa according to the method of claim 21 and inseminating a mammal with the treated sample containing a population of spermatozoa.

29 (Withdrawn). The method of claim 28, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

30 (Withdrawn). The method of claim 28, wherein the semen is cooled to at least about 16°C.

31 (Withdrawn). The method of claim 28, wherein the semen is cooled to at least about 12°C.

32 (Withdrawn). A method of increasing the percentage of mammalian offspring of a predetermined sex, the method comprising:

cooling a specimen of semen containing a population of cells to a predetermined temperature;

waiting until a predetermined percent of the cells exhibit a punctate pattern, which is capable of determination by labeling the sperm cells with Koo antibody;

contacting the sample with a cell binding agent to preferentially bind to a selected population of cells for a time sufficient for the cell binding agent to bind the selected cells; and



separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells; and

administering at least a portion of the treated sample to the reproductive tract of a female mammal.

33 (Withdrawn). The method of claim 32, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 10% greater than a second number of cells exhibiting a non preferred sex type.

34 (Withdrawn). The method of claim 32, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 20% greater than a second number of cells exhibiting a non preferred sex type.

35 (Withdrawn). The method of claim 32, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 50% greater than a second number of cells exhibiting a non preferred sex type.

36 (Withdrawn). The method of claim 32, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 100% greater than a second number of cells exhibiting a non preferred sex type.

37 (Withdrawn). The method of claim 32, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

38 (Withdrawn). The method of claim 32, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.



39 (Withdrawn). The method of claim 32, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

40 (Withdrawn). The method of claim 32, wherein the semen is cooled to at least about 16°C.

41 (Withdrawn). The method of claim 32, wherein the semen is cooled to at least about 12°C.

42 (Withdrawn). A method for fractionating an ejaculate of a mammal, the method comprising:

- obtaining an ejaculate;
- cooling the ejaculate to a predetermined temperature;
- waiting for a period of time between about 2 hours and about 24 hours after collection of the ejaculate; and
- fractionating the ejaculate to obtain a selected population of cells.

43 (Withdrawn). The method of claim 42, wherein the selected population of cells comprises a higher number of X-chromosome bearing sperm relative to Y-chromosome bearing sperm and the second component comprises a higher number of Y-chromosome bearing sperm relative to X-chromosome bearing sperm.

44 (Withdrawn). The method of claim 42, wherein the fractionating step comprises contacting the ejaculate with a cell binding agent; permitting the sperm of a non selected sex type to preferentially bind to the cell binding agent, and separating the cell binding agent with preferentially bound sperm of the non selected sex type from non bound sperm, thereby providing a selected population of cells.

45 (Withdrawn). A method of insemination, the method comprising obtaining the selected population of cells according to the method of claim 44 and inseminating a mammal with the selected population of cells.



46 (Withdrawn). The method of claim 45, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

47 (Withdrawn). The method of claim 45, wherein the semen is cooled to at least about 16°C.

48 (Withdrawn). The method of claim 45, wherein the semen is cooled to at least about 12°C.

49 (Withdrawn). The method of claim 42, wherein the fractionating step is carried out between about 2 hours and about 12 hours after collection of the ejaculate.

50 (Withdrawn). The method of claim 42, wherein the fractionating step is carried out between about 4 hours and about 8 hours after collection of the ejaculate.

51 (Withdrawn). The method of claim 42, wherein the fractionating step is carried out at about 6 hours after collection of the ejaculate.

52 (Withdrawn). The method of claim 42, wherein the semen is cooled to at least about 16°C.

53 (Withdrawn). The method of claim 42, wherein the semen is cooled to at least about 12°C.

54 (Withdrawn). A method of insemination, the method comprising obtaining semen containing a population of spermatozoa according to the method of claim 42 and inseminating a mammal with the selected population of cells.

55 (Withdrawn). The method of any of claim 54, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.



56 (Withdrawn). The method of claim 54, wherein the semen is cooled to at least about 16°C.

57 (Withdrawn). The method of claim 54, wherein the semen is cooled to at least about 12°C.

58 (Withdrawn). The method of claim 42, wherein the fractionating step comprises incubating the ejaculate with a cell binding agent; permitting the sperm of a non selected sex type to preferentially bind to the cell binding agent; and separating the cell binding agent with preferentially bound sperm of the non selected sex type from non bound sperm, thereby providing a selected population of cells, the cell binding agent comprising a bead having a surface treatment comprising a material that preferentially binds to sperm of a non selected sex.

59 (Withdrawn). The method of claim 42, wherein the fractionating step comprises incubating a mixture of the ejaculate with a cell binding agent; permitting the sperm of a non selected sex type to preferentially bind to the cell binding agent; adding beads to the mixture, the beads being capable of binding with the cell binding agent; incubating the beads with the mixture to bind to the cell binding agent; and separating the beads and cell binding agent thereby preferentially removing sperm of the non selected sex type from the mixture, preferentially leaving sperm of a selected sex type.

60 (Currently Amended). A method for treating a specimen of semen containing sperm cells to increase the relative number of sperm cells of a preferred sex type in a treated specimen to increase the potential for conceiving an offspring of the preferred sex, the method comprising separating the semen into two components comprising a first component having a higher number of sperm of the preferred sex type than sperm of a non preferred sex type and a second component having a higher number of sperm of the non preferred sex type relative to sperm of the preferred sex type, wherein the separating step is performed in a window of time that can be determined by locating a maximum in the  $\alpha$  curve obtained by plotting percent female cells determined by FISH against percent Koo positive cells for aliquots of a semen sample taken at various times



after collection, determining the time at which the maximum percent female cells occurs, and beginning the separation step no earlier than about one hour before the time of the maximum percent female cells.

61 (Original). The method of claim 60, wherein the separating step comprises contacting the sperm with a cell binding agent; permitting the sperm of the non preferred sex type to preferentially bind to the cell binding agent, and separating the cell binding agent with preferentially bound sperm of the non preferred sex type from non bound sperm, thereby providing said first component.

62 (Original). The method of claim 60, wherein the first component comprises a higher number of X-chromosome bearing sperm relative to Y- chromosome bearing sperm and the second component comprises a higher number of Y- chromosome bearing sperm relative to X- chromosome bearing sperm.

63 (Original). The method of claim 60, further comprising cooling the semen before performing the separating step.

64 (Original). The method of claim 60, further comprising collecting the semen and immediately cooling the semen.

65 (Original). The method of claim 64, wherein the semen is cooled to at least about 16°C.

66 (Original). The method of claim 64, wherein the semen is cooled to at least about 12°C.

67 (Currently Amended). A method for increasing the probability of producing an offspring of a mammalian species having a preferred sex, said method comprising: separating semen containing sperm cells of the mammalian species into two components comprising a first component having a higher number of sperm of the preferred sex type than sperm of a non preferred sex type and a second component



having a higher number of sperm of the non preferred sex type relative to sperm of the preferred sex type, wherein the separating step is performed in a window of time that can be determined by locating a maximum in the a curve obtained by plotting percent female cells determined by FISH against percent Koo positive cells for aliquots of a semen sample taken at various times after collection, determining the time at which the maximum percent female cells occurs, and beginning the separation step no earlier than about one hour before the time of the maximum percent female cells.

68 (Original). The method of claim 67, wherein the separating step comprises contacting the sperm with a cell binding agent; permitting the sperm of the non preferred sex type to preferentially bind to the cell binding agent, and separating the cell binding agent with preferentially bound sperm of the non preferred sex type from non bound sperm, thereby providing said first component.

69 (Original). The method of claim 67, wherein the first component comprises a higher number of X-chromosome bearing sperm relative to Y- chromosome bearing sperm and the second component comprises a higher number of Y- chromosome bearing sperm relative to X- chromosome bearing sperm.

70 (Original). The method of claim 67, further comprising cooling the semen before performing the separating step.

71 (Original). The method of claim 67, further comprising collecting the semen and immediately cooling the semen.

72 (Original). The method of claim 71, wherein the semen is cooled to about 12°C.

73 (Original). The method of claim 69, wherein the first component contains two-thirds viable X- chromosome bearing sperm and one-third viable Y - chromosome bearing sperm.



74 (Original). The method of claim 67, wherein twice as many female calves are born than male calves when the treated semen is used to fertilize a plurality of eggs.

75 (Currently Amended). A method for separating a selected population of cells from a sample of semen, the method comprising:

cooling the semen to a predetermined temperature;

waiting for a time that can be determined by locating a maximum in the a curve obtained by plotting percent female cells determined by FISH against percent Koo positive cells for aliquots of a semen sample taken at various times after collection, determining the time at which the maximum percent female cells occurs, and beginning the following step no earlier than about one hour before the time of the maximum percent female cells;

contacting the sample with a cell binding agent to preferentially bind to the selected population of cells for a time sufficient for the cell binding agent to bind the selected cells; and

separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells.

76 (Original). The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 10% greater than a second number of cells exhibiting a non preferred sex type.

77 (Original). The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 20% greater than a second number of cells exhibiting a non preferred sex type.

78 (Original). The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 50% greater than a second number of cells exhibiting a non preferred sex type.



79 (Original). The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 100% greater than a second number of cells exhibiting a non preferred sex type.

80 (Original). The method of claim 75, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

81 (Original). The method of claim 75, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.

82 (Original). A method of insemination, the method comprising obtaining semen containing a population of spermatozoa according to the method of claim 75 and inseminating a mammal with the treated sample containing a population of spermatozoa.

83 (Original). The method of claim 82, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

84 (Original). The method of claim 82, wherein the semen is cooled to at least about 16°C.

85 (Original). The method of claim 82, wherein the semen is cooled to at least about 12°C.

86 (Currently Amended). A method of increasing the percentage of mammalian offspring of a predetermined sex, the method comprising:  
cooling a specimen of semen containing a population of cells to a predetermined temperature;  
waiting for a time that can be determined by locating a maximum in the a curve obtained by plotting percent female cells determined by FISH against percent Koo



positive cells for aliquots of a semen sample taken at various times after collection, determining the time at which the maximum percent female cells occurs, and beginning the following step no earlier than about one hour before the time of the maximum percent female cells;

contacting the sample with a cell binding agent to preferentially bind to a selected population of cells for a time sufficient for the cell binding agent to bind the selected cells;

separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells; and

administering at least a portion of the treated sample to the reproductive tract of a female mammal.

87 (Original). The method of claim 86, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 10% greater than a second number of cells exhibiting a non preferred sex type.

88 (Original). The method of claim 86, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 20% greater than a second number of cells exhibiting a non preferred sex type.

89 (Original). The method of claim 86, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 50% greater than a second number of cells exhibiting a non preferred sex type.

90 (Original). The method of claim 86, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 100% greater than a second number of cells exhibiting a non preferred sex type.



91 (Original). The method of claim 86, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

92 (Original). The method of claim 86, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.

93 (Original). The method of claim 86, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

94 (Original). The method of claim 93, wherein the semen is cooled to at least about 16 °C.

95 (Original). The method of claim 93, wherein the semen is cooled to at least about 12 °C.